

Effects of soil amendment treatments on survival and growth of four fast-growing tree species on
degraded mine areas in the Peruvian Amazon

by

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ABSTRACT OF THE PROJECT OF

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This paper aims to increase the knowledge on establishment practices for four fast-growing species (*Guazuma crinita* Mart., *Eucalyptus grandis* x *E. urophylla*, *Bixa orellana* L., *Psidium guajava* L.) in areas degraded by gold mining in the Peruvian Amazon. The study was carried out in Madre de Dios region, located in the southeast of Peru, in sites degraded by intensive mining. The study, that started in 2017, aims to quantify the effects of biochar and fertilizers on seedling growth and survival. The treatments tested were control, biochar, enriched biochar (biochar enriched with efficient microorganisms and fertilizers), and organic liquid fertilizer plus N-P-K.

After three growing seasons, strong differences are shown in the survival of the four species studied, but no effect of soil treatments on growth increased. *Psidium guajava* showed the highest survival rate and *Eucalyptus grandis* x *E. urophylla* showed the larger growth. No treatments by species effect were observed on seedling growth and survival. Further research is needed to provide better alternatives to increase seedling survival and growth in areas degraded by mining in the Peruvian Amazon.

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*Soil amendments in this Capstone Project include biochar and organic waste plus factory-produced N-P-K and other fertilizers. This definition complements the widely used, local Spanish translation, enmienda; it includes all potential soil additions to promote survival and growth of trees and edible crops.

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1. Introduction

Peru is the country with the second-largest extension of Amazonian forests in South America. Approximately 60% of the national territory is covered by forests with a total extension of 82.5 million hectares (MINAM, 2016). Tropical forest ecosystems provide varied goods and services, such as timber/non-timber products and ecosystem services. Some of these significant ecosystem services include cleaning air, improving water quality, stabilizing soil, and regulating the climate (Wilkinson et al., 2014). However, there are current environmental degradation factors that are affecting its sustainability.

Peruvian forests are threatened by deforestation and degradation processes leading to a loss of biodiversity. According to MINAM (2016), an average of 120,000 ha of forest lands are lost every year due to land use change for agriculture, illegal timber extraction and mining. In the western Amazon, over a period of 34 years, more than 100,000 ha have been deforested due to artisanal-scale gold mining activities (Caballero et al., 2018). So, it is necessary to develop reforestation strategies for these areas. This project is focused on areas that have been deforested due to mining activities.

1.1. Research objective

This paper analyzes the effects of different treatments of nutrient additions on early growth and survival of four fast-growing tree species (including timber and non-timber species) planted in degraded soils in the Madre de Dios region of the Peruvian Amazon. The overall aim is to contribute to the knowledge on stand establishment practices implemented to increase seedling survival and growth in reforestation projects with timber and non-timber species in areas degraded by mining in the Peruvian Amazon. It will provide useful information for forest managers on sustainable alternatives for seedling establishment on land recovery projects.

Specific objectives:

- i. Quantify the effects of biochar, enriched biochar, and fertilizers without biochar on survival and growth of seedlings of four fast-growing tree species (*Guazuma crinita* Mart., *Eucalyptus grandis* x *E. urophylla*, *Bixa orellana* L., *Psidium guajava* L.) planted on degraded soils in the Madre de Dios Region in Peruvian Amazonia.
- ii. Quantify the interactive effects between the soil amendment treatments and the species tested.

2. Background

2.1. Reforestation context

The study area is located in the Madre de Dios region in southeastern Peru. Although alluvial gold mining is considered an important economic activity in this region, it has also generated significant negative socio-environmental impacts due to inadequate harvesting practices (Cabanillas et al., 2019 and Chirif et al., 2021). Gold mining has increased significantly in recent decades and can be considered an important driver of forest degradation and environmental contamination in the Madre de Dios region. According to the Centro de Innovación Científica Amazónica (CINCIA for its acronym in Spanish, 2018), 9,860 ha were deforested in 2017, making this figure the highest historical record of forest cover loss due to gold mining in Peru. That loss represented about 38% of the total deforested area in Madre de Dios, and 6.9 % of the total deforested area in Peru (CINCIA, 2018).

There is a gap of knowledge on the best reforestation activities for those degraded areas aforementioned. These reforestation activities should be designed to ensure the success the establishment of the desired forest vegetation cover, which, when applied on degraded areas by mining, can improve the physical, chemical, and biological properties of the soil, leading to higher

soil quality when compared to degraded areas without forest vegetation cover (Paulucio et al., 2017). Furthermore, reforestation in post-mining areas should consider specific habitat requirements, including them into "targeted production of planting material" (Karličić et al., 2016). Species selection and seedling quality are critical components in ensuring a successful forest restoration program (Grossnickle and MacDonald, 2018). In the same way, improvement of reforestation techniques, such as nutritional amendments, and the monitoring of stand development, should be considered in the management plan in this type of reforestation projects.

2.2. Soil nutrient bioavailability in mining-degraded sites

There are several soil factors that could influence the process of seedling establishment. Ferraz (1993) pointed out that low levels of soil organic matter and some essential nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) could reduce the growth of trees planted in reforestation in mining sites in Amazonia. In addition to poor soil fertility, the presence of mercury (Hg) in plant tissues has been associated to reduced growth in areas degraded by gold mining, indicating that remediation and restoration are subjected to great challenges under those conditions (Román-Dañobeytia et al., 2015). Therefore, increasing and improving our knowledge on the influences of these factors is key for developing strategies for the establishment and management of reforestation projects in mining-degraded areas.

2.3. Biochar

Biochar (biomass-derived char) is organic matter thermochemically decomposed in the absence or low of oxygen concentrations during the pyrolysis process (Marjenah et al., 2016) producing a solid and stable material with high carbon content (Yang et al., 2021). This form of charcoal can be made from a variety of biomass feedstock, such as crop residues, wood chips, rice husk, among other solid wastes (Xie et al., 2014 and Marjenah et al., 2016).

Biochar can improve soil conditions by increasing nutrient and water retention and availability (Glaser et al., 2002 and Sessions et al., 2019). These improvements could benefit plant growth because the biochar particles have a larger surface area that could allow them to absorb nutrients and promote the growth of beneficial bacteria or fungi (Marjenah et al., 2016). In addition to its high specific surface area, Xie et al. (2014) mention that properties like microporosity and adsorption capabilities may provide great potential for soil amendment and environmental remediation. For in situ remediation of mercury-contaminated sites, biochar is recognized as a functional material because it may decrease the mobility of inorganic mercury (Hg) in soils (Yang et al., 2021). An example of this property was studied by Shu et al. (2016) demonstrating that biochar amendment in mercury-mined soils could reduce methylmercury (MeHg) levels in rice plants.

High-quality, low-cost biochar production can greatly increase the success of reforestation/afforestation projects by improving plant performance (CINCIA, 2019 and Lefebvre et al., 2019). According to Xie et al. (2014) the quality is strongly influenced by the production method and feedstock composition. Regarding the production method or technology, this could also influence costs and benefits of biochar. These cost considerations could influence the profitability for larger scale utilization by land managers (Crane-Droesch et al., 2013).

2.4. Efficient microorganisms

Efficient microorganisms (EM-1®) are a concentrate of beneficial microorganisms contained in a dormant state used for the recovery and biological activation of soils (Biopunto, 2020). Among its main benefits are the improvement of soil physical, chemical, and biological conditions, and the reduction of salinity and heavy metals concentration (Biopunto, 2020). According to de Araujo et al. (2021), efficient microorganisms would produce organic acids and plant hormones that may have direct or indirect influences on plant growth.

Improving plant growth and crop productivity can be one of the main influences of using EM. Calero et al. (2019) found that soil inoculation with EM can provide better results in increasing bean crop productivities. In addition to increasing crop yield and quality, the inoculation of efficient microbial communities to the soil can also improve the quality and health of the soil and plant growth resulting in potential benefits in the restoration of wasteland (Singh et al., 2011). Therefore, the response of soil treated with EM may be related to neutralizing the effects of heavy metals in remediation processes of contaminated soils (de Araujo et al., 2021). The study of the possible beneficial effects on tree species still requires further research that could provide more evidence of their potential for restoration projects.

2.5. Organic liquid fertilizer

Organic liquid fertilizer (OLF), or commonly called “biol”, is the liquid fraction resulting from the anaerobic decomposition occurring in a biodigester (Gomero, 2005). It is a homemade fertilizer produced from the decomposition of animal manure or vegetable compost and can be applied directly to the soil or foliarly (Cabanillas and Alférez, 2021). The composition of biol can provide important elements for plant growth (nitrogen, phosphorus, potassium, and calcium) as well as regulatory enzymes (auxins and gibberellins) that stimulate its development (Gomero, 2005). For these reasons, biol can be used in plants as a fertilizer. The use of organic liquid fertilizer can also have a positive effect on the recovery of soils contaminated with metals as pointed out by Chiang et al. (2016), who after doing a comparative study of solutions prepared with OLF could increase fertility indices (nitrogen, phosphorus, and potassium) after its use.

2.6. Socioeconomic context

The remediation or reclamation of landscapes post-mining activities may involve multiple stakeholders. The articulation of these different groups (government, mining companies,

indigenous groups, local communities, and environmental organizations) could configure the pursuit of practices that can sustain healthy ecosystems (Beckett and Keeling, 2019). When O'Brien et al. (2020) analyzed remediation projects in developing countries and found that effective participation of different stakeholder groups, from the early stages of the project, experienced success due to the creation of shared goals compared to those remediation projects that had difficulties in enabling active participation of local stakeholders. Therefore, critical thinking beyond biophysical aspects should be considered important in landscape remediation.

The involvement of local communities in reforestation and agroforestry programs could be highly recommended for their sustainability, as this articulated work could also allow community members to choose tree species that have economic, cultural, and natural value for them. For example, as pointed out by Labarta and Weber (1998), fast-growing and low-density timber species contributes significantly to farmers' income and improvement of livelihoods. This could strengthen the added value of forest land and shift towards sustainable economic activities.

3. Methods

3.1. Study site

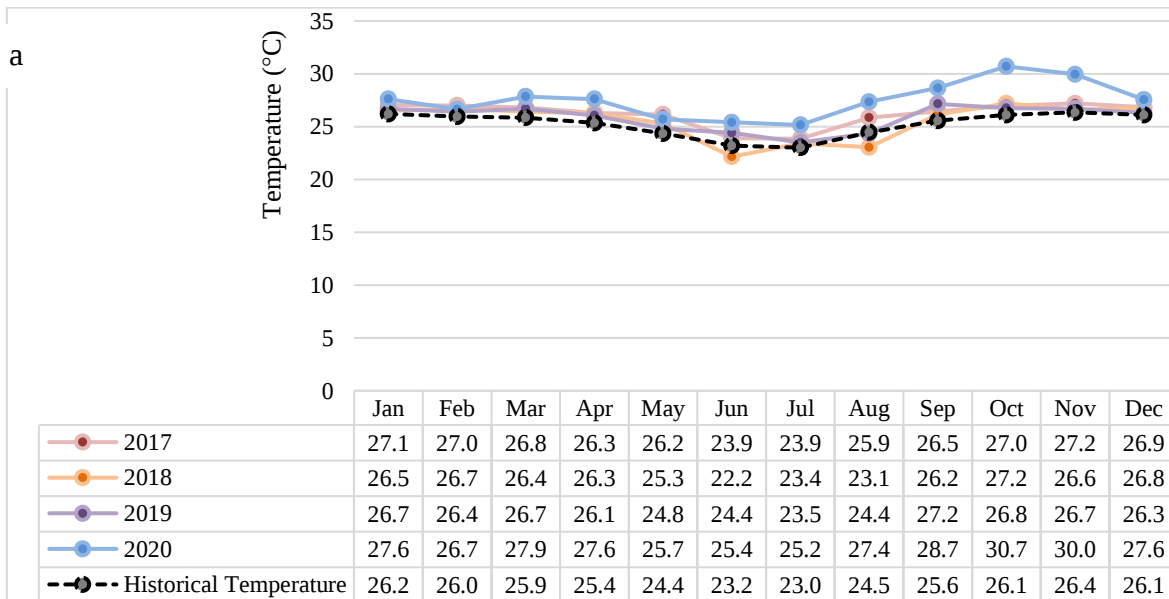
This study was conducted in the area of the native community of San Jacinto (12°36'16.5"S 69°23'15.4"W), located in the district and province of Tambopata in the Madre de Dios region in Peru (Figure 1). Soil analysis conducted by CINCIA, show that the soil has a highly sandy texture with an organic matter of 0.12 ± 0.09 % (average $\pm$ standard deviation), pH of 5.29 ± 0.37 , a CEC of 4.68 ± 0.62 Cmol (+)/kg, and mercury (Hg) presence of 0.02 ± 0.01 mg/kg.

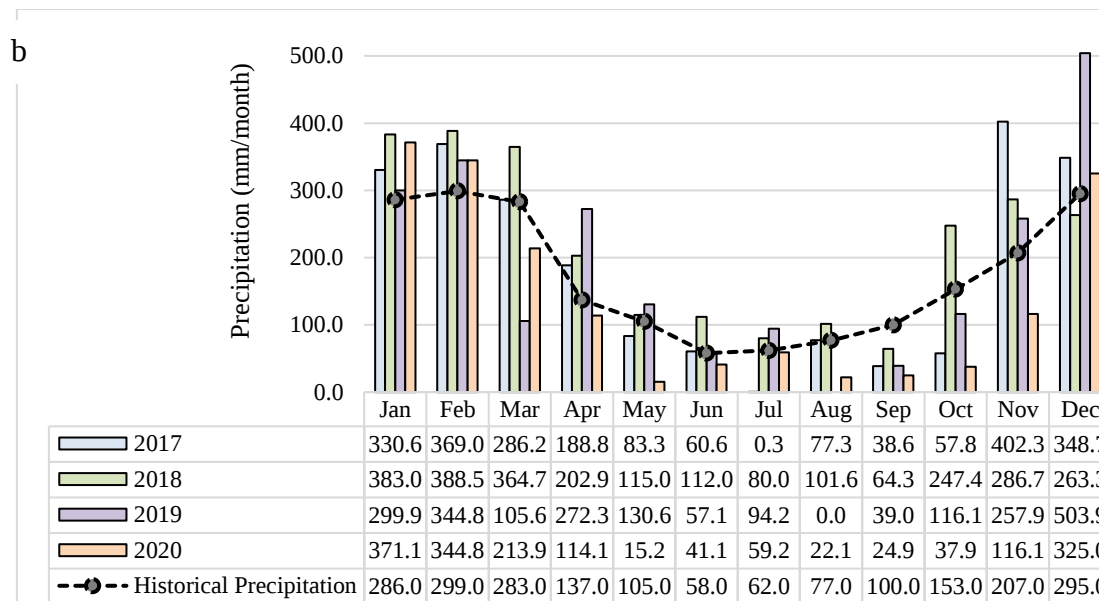
Figure 1. Images showing a) location of the experimental plots, and b) site view before planting.



The study site, located at an altitude of 198 meters above sea level, had an average annual temperature of 27.2°C, and an average monthly rainfall of 182 mm per month. Figure 2 shows monthly average temperature (a) and total rainfall (b) during the evaluation period (2017-2020), including historic means (Servicio de Meteorología Aeronáutica- CORPAC, 2022).

Figure 2. Monthly average temperature (a) and monthly total precipitation (b) during the evaluation period 2017-2020 (upper panel). Circles represent mean historic values.





3.2. Study design

Twelve experimental plots were installed in the study area, which included three replicates of each treatment. The study plots had an area of 0.39 ha in which approximately 400 seedlings were planted. The original plots included 28 different species, from which four species were selected for this study, where two were classified as timber species and two as non-timber species. The soils where each seedling was installed were characterized. These soils were mainly soils without vegetation cover, approximately for 85% of the seedlings. The rest of the seedlings were installed in soils with gravel mining accumulation and areas with presence of natural regeneration.

The timber species selected were *Guazuma crinita* Mart. ("bolaina blanca") and *Eucalyptus grandis* x *E. urophylla* ("eucalyptus"). *Guazuma crinita* is a fast-growing Amazonian timber species that is generally found in second-growth forests and can be harvested 8 to 9 years after planting (Flores, 2007). Due to its multiple uses and solid establishment in national markets, it is considered as a species with great potential for sustainable forest management by small producers (Putzel et al., 2013). *Eucalyptus* x *urograndis* is a hybrid species coming from the crossing of

Eucalyptus grandis W. Hill and *Eucalyptus urophylla* S.T. Blake (Barja et al., 2001). Attributes of both species of eucalyptus provide to the hybrid characteristics of rapid growth and greater resistance to limiting conditions such as climate and diseases (Kullan et al., 2012).

The non-timber species selected were *Bixa orellana* L. (“achiote”) and *Psidium guajava* L. (“guayaba”). *Bixa orellana* is a shrub or small tree of 1-3 meters height with abundant foliage that is highly cultivated in the Amazon for its fruits (Reynel et al., 2016). According to Vilar et al. (2014), the dyes found in its seeds have wide commercial application, so it is considered of industrial importance, as well as having multiple cultural uses. *Psidium guajava* is also a shrub or small tree 2-5 meters height (Reynel et al., 2016). Its fruits, wood, and leaves, in addition to medicinal uses, are used for food, house construction and carpentry (Gutiérrez et al., 2008).

The soil amendment treatments (hereinafter referred to as soil treatments) applied were four: control (T1), biochar (T2), enriched biochar (T3) and organic liquid fertilizer plus NPK (T4). Biochar enriched refers to biochar enriched with efficient microorganisms and fertilizers (described below). Each of the treatments were applied during the installation process of the seedling selected for the experiment on November 15, 2017 (planting date). The biochar used in treatments T2 and T3 was produced locally from Brazil nut (*Bertholletia excelsa*) husk residues, using a Top-Li Up-Draft type pyrolizer drum (CINCIA, 2019). The main characteristics of the biochar are shown in Table 1. The molar H/Corg ratio refers to the degree of carbonization, and consequently, of the stability of biochar (Schmidt et al., 2016).

Efficient microorganisms (EM-1®) are a liquid concentrate with a density of 1.02 g cm⁻³ and pH ranging between 3.0-3.6. Its composition consists of purified water, sugarcane mixture, lactic acid bacteria (*Lactobacillus* spp), photosynthetic bacteria (*Rhodopseudomonas* spp) and yeasts (*Saccharomyces* spp).

Table 1. Main characteristics of biochar used in this study (from Lefebvre et al., 2019).

Biochar Parameter	Unit	Value
Bulk density	g cm^{-3}	0.486
Specific surface	$\text{m}^2 \text{g}^{-1}$	147.6
Ash content (550 °C)	%	7.8
pH	-	9.6
Carbon (C)	%	87.6
Total Nitrogen (N)	%	1.07
H/C _{org} ratio (molar)	-	0.19
Calcium (Ca)	%	0.5
Magnesium (Mg)	%	0.3
Potassium (K)	%	2.1
Phosphorus (P)	%	0.3

For T2 and T3, the amount of biochar applied was 1 kg per seedling. For T3, the biochar was mixed with efficient microorganisms (EM) + 200 ml organic liquid fertilizer + 45 g of NPK 20-20-20 (MOLIMAX® compound fertilizers, Molinos & Cía fertilizantes, Peru). The mixture of biochar with EM was made at a proportion of 20 liters of EM per ton of biochar. For T4, which corresponds to T3 without biochar, a mix of 200 ml organic liquid fertilizer + 45 g of NPK 20-20-20 (MOLIMAX® compound fertilizers, Molinos & Cía fertilizantes, Peru) was applied to each seedling. All treatments were applied directly to the soil at planting time.

The monitoring and management were carried out every three months after the experimental plots were installed. A total of 4 supervisions per year were carried out. Weed control was carried out mechanically using string trimmer. Additionally, all plots (including control) received at a dose of fertilization (200 ml of biol + 45 g of NPK/plant) 6 months after planting. This fertilizer application followed the post-planting management plan defined by CINCIA as a protocol of methods to generate conditions for their viability (CINCIA, 2021).

All seedlings were produced in the nursery of CINCIA located in the Mazuko city in the district of Inambari, Tambopata province of Madre de Dios region. This nursery had a plant production system using deepot cells (Stuewe & Sons, 2022) with an automatic irrigation system (Cabanillas et al., 2019) (Figure 3). The seedlings used for the installation of the experimental plots

corresponded to the first production period of the nursery started in June 2017. The production process is detailed in general for the entire production by Cabanillas et al. (2019). The substrate was a mixture of agricultural soil, sawdust, and moss in quantities of 20%, 50% and 30%, respectively.

Table 2. Average seedling height (cm) and ground line diameter (mm) at planting time.

Species	Height (cm)	Diameter (mm)
<i>Guazuma crinita</i>	48	4
<i>Eucalyptus urograndis</i>	82	5
<i>Bixa orellana</i>	59	5
<i>Psidium guajava</i>	47	4

The period of permanence in the nursery was approximately 5 months. During the first 2 months, priority was given to seed germination and initial seedling growth, receiving phosphorus (P) fertilization to promote root development. During this period seedlings were growing under 80% shade cover. The following three months were focused on the rapid growth and hardening phase, light exposure was increased to 50%, having daily irrigation and fertilization with N-P-K. After seedlings reached target height and diameter size (Table 2), all plants were transported to the field for planting in November 2017.

Figure 3. Images showing a) greenhouse used for seedling production in Mazuko city, and b) example of a seedling of *Bixa Orellana* into depot cell container in the greenhouse (from CINCIA, 2018)



In the field, 12 rows (plots) were randomly assigned to the four soil treatments (three replicates per treatment). Within each row (plot), seedlings from all species were randomly planted at the beginning of the rainy season (November 2017). These were planted at a spacing of 3 m between plants and 3 m between rows. The number of seedlings of each species per plot was not uniform, ranging between 16 (*Eucalyptus x urograndis*, Plot 11) and 34 (*Psidium guajava*; Plot 3). A detail of number of seedlings per plot is shown in Table 3.

Table 3. Number of seedlings planted per plot by species and treatments.

Species	P1 T4	P2 T1	P3 T4	P4 T3	P5 T1	P6 T2	P7 T2	P8 T1	P9 T3	P10 T2	P11 T4	P12 T3
<i>Guazuma crinita</i>	20	20	21	23	18	19	21	19	20	20	18	21
<i>Eucalyptus urograndis</i>	30	26	25	20	24	26	28	29	27	25	16	24
<i>Bixa orellana</i>	24	24	21	21	24	25	24	26	23	22	19	25
<i>Psidium guajava</i>	31	33	34	31	33	31	32	36	29	32	23	32

P: Plot identifier (P1-P12); T: Treatment identifier (T1-T4).

The experimental design is split-plot design with whole-plot-factor in a complete randomize design, where the whole-plot factor is the soil treatment, and the sub-plot is the species. All living seedlings of selected species were evaluated during years 2017 (November, date of planting), 2019 (March, age 1.3 years after planting) and 2020 (November, age 3 years after planting). Measurements included seedling ground line, diameter (D, cm), height (H, cm) and survival (S, %). The stem volume index (D^2H ; dm^3) was computed for each measured seedling using D and H expressed in dm.

3.3. Data analysis

The assumption that the errors are normally distributed was verified by the Anderson-Darling normality test and homogeneity of variances. Analysis of variance, including Tukey HSD (Honestly significant difference) adjustments, was performed using the mixed procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to test fixed treatment effects on tree size and survival

while including the random effect of species within treatment plot. The mean value of all seedlings of each species within each soil treatment plots was used for all ANOVA tests. Survival data was normalized using arcsin (logit) transformation. All figures were produced using SigmaPlot version 14 (Systat Software, Inc. San Jose, CA).

4. Results

A summary of the effects of species and all treatments on H, D, D²H and S observed at last measurement (age 3 years after planting) is shown in Table 4. There was a strong effect of species for all response variables ($P < 0.021$). However, there were no significant treatment and species x treatment effects for any response variable ($P > 0.096$).

Table 4. P-values from ANOVA tests for the effects of species (Spp), soil amendment treatments (Trt) and their interaction on seedling height (H), ground line diameter (D), stem volume index (D²H) and Survival (S) at age 3 years. Significant effects are denoted in bold.

Source	H	D	D ² H	S
Spp	0.0063	0.002	0.0211	0.0001
Trt	0.1505	0.096	0.1325	0.8319
Spp x Trt	0.8715	0.997	0.9887	0.9642

Table 5 shows the summary of mean values of the response variables (H, D, D²H, and S) for each species and soil treatments. When comparing soil amendment treatments within each species, no effect was observed for response variables. However, as show in Table 4, there were significant differences between species. On average, *Psidium guajava* showed a higher S compared to the other three species ($P = 0.0001$). Additionally, *Eucalyptus x urograndis* showed, on average, higher H ($P = 0.0038$), D ($P = 0.0139$) and D²H ($P = 0.0141$) than *Guazuma crinita*.

Table 5. Mean height (H, cm), ground line diameter (D, cm), stem volume index (D²H, dm³), Survival (S, %), and average values (AVG) of each response variables at age 3 years for *Guazuma crinita*, *Eucalyptus x urograndis*, *Bixa orellana* and *Psidium guajava* seedlings growing under different soil treatments. Values in parenthesis represent standard error.

Species	Treatment	H (cm)	D (cm)	D ² H (dm ³)	S (%)
<i>Guazuma crinita</i>	T1	215.6 (44.1)	2.7(0.4)	2.1 (0.7)	10.7 (3.4)
	T2	207.9 (48.7)	3.2 (0.3)	4.4 (1.6)	22.1 (8.0)
	T3	242.5 (20.9)	3.5 (0.6)	5.1 (2.2)	27.3 (13.7)
	T4	208.8 (55.4)	2.8 (0.6)	4.8 (2.1)	33.1 (10.4)
	AVG	218.7	3.1	4.1	23.3
<i>Eucalyptus x urograndis</i>	T1	288.0 (13.7)	4.0 (0.2)	8.2 (1.1)	27.1 (5.0)
	T2	292.0 (33.7)	4.3 (0.4)	7.9 (2.4)	41.4 (14.6)
	T3	362.0 (28.2)	4.6 (0.7)	11.3 (3.5)	35.2 (8.1)
	T4	249.3 (35.3)	3.8 (0.6)	6.8 (3.3)	32.3 (12.2)
	AVG	297.8	4.2	8.5	34.0
<i>Bixa orellana</i>	T1	224.4 (10.4)	4.0 (0.5)	5.0 (1.2)	38.0 (10.3)
	T2	254.4 (10.3)	4.7 (0.3)	6.6 (0.4)	29.6 (10.8)
	T3	245.4 (9.6)	5.2 (0.8)	9.0 (2.5)	39.7 (8.3)
	T4	246.5 (11.0)	4.1 (0.1)	5.7 (0.3)	37.9 (17.9)
	AVG	242.7	4.6	6.6	36.3
<i>Psidium guajava</i>	T1	257.1 (9.1)	3.6 (0.1)	4.9 (0.2)	64.0 (8.9)
	T2	249.9 (19.3)	3.6 (0.4)	5.0 (1.1)	67.5 (13.6)
	T3	283.9 (21.5)	4.4 (0.3)	7.2 (1.5)	63.0 (2.6)
	T4	238.2 (42.2)	3.8 (0.7)	5.0 (2.4)	62.9 (10.6)
	AVG	257.3	3.9	5.5	64.1

4.1. Survival

The time series of average S for each species and soil treatment are shown in Figure 4. In general, a reduction in S was observed after the first growing season. At age 3 years after planting, *Psidium guajava* showed the highest survival ($P < 0.0001$), averaging 64.1%, while *Bixa orellana*, *Eucalyptus x urograndis* and *Guazuma crinita* averaged 36.3, 34.0 and 23.3%, respectively. Most of the mortality was observed during the first year after planting, reaching survival of 44.1, 43.7, 51.4 and 69.2%, for *Guazuma crinita*, *Eucalyptus x urograndis*, *Bixa orellana* and *Psidium guajava*, respectively (Table 5; Figure 4).

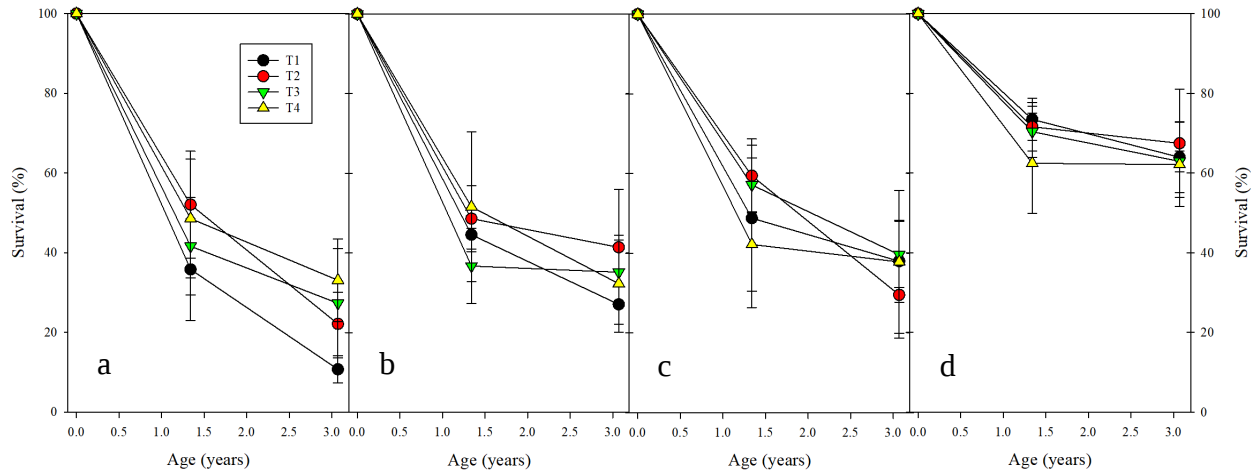


Figure 4. Time series of survival (%) for a) *Guazuma crinita*, b) *Eucalyptus x urograndis*, c) *Bixa orellana*, and d) *Psidium guajava* growing under different soil treatments. Error bars represent standard error.

4.2. Height

The time series of average H for each species and soil treatment are shown in Figure 5. At year 3 after planting, *Eucalyptus x urograndis* showed higher H than *Guazuma crinita* ($P=0.0038$). No other difference was observed across species. Even though no significant effect was found for soil treatments, a general trend was observed for seedlings growing under T3, having a gain in H of 26.9, 74.0 and 26.8 cm, for *Guazuma crinita*, *Eucalyptus x urograndis*, and *Psidium guajava*, respectively, when compared to seedlings growing in T1 plots (Table 5; Figure 5).

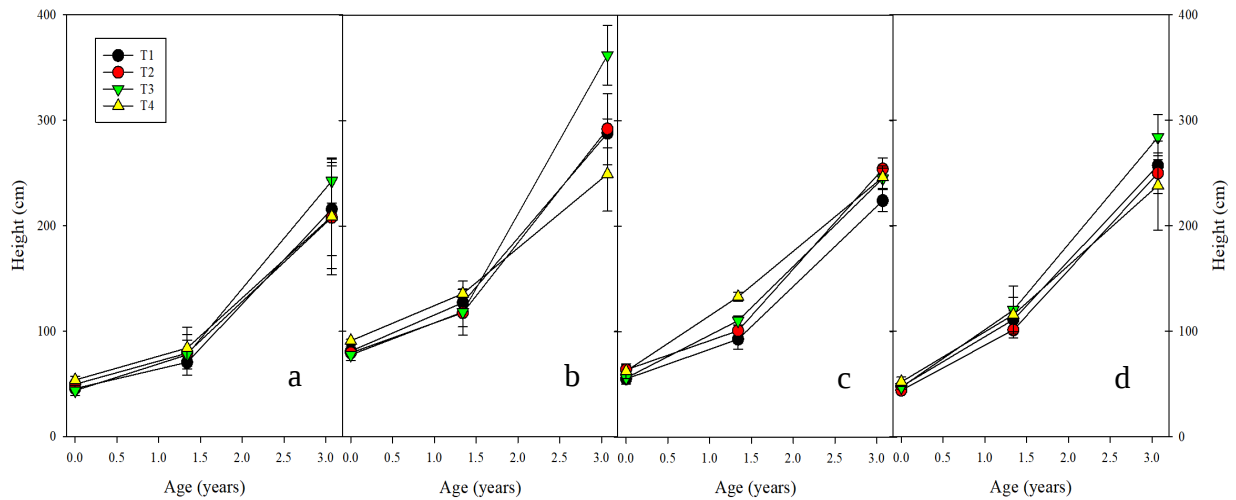


Figure 5. Time series of height (cm) for a) *Guazuma crinita*, b) *Eucalyptus x urograndis*, c) *Bixa orellana*, and d) *Psidium guajava* growing under different soil treatments. Error bars represent standard error.

4.3. Ground line diameter

The time series of average D for each species and soil treatment are shown in Figure 6. At year 3 after planting, *Bixa orellana* and *Eucalyptus x urograndis* showed higher D than *Guazuma crinita*, ($P= 0.0016$ and 0.0139 , respectively). Similar to H, even though no significant effect was found for soil treatments, a general trend was observed for seedlings growing under T3, having a gain in D of 0.8, 0.6, 1.2 and 0.8 cm, for *Guazuma crinita*, *Eucalyptus x urograndis*, *Bixa orellana* and *Psidium guajava*, respectively, when compared to seedlings growing in T1 plots (Table 5; Figure 6).

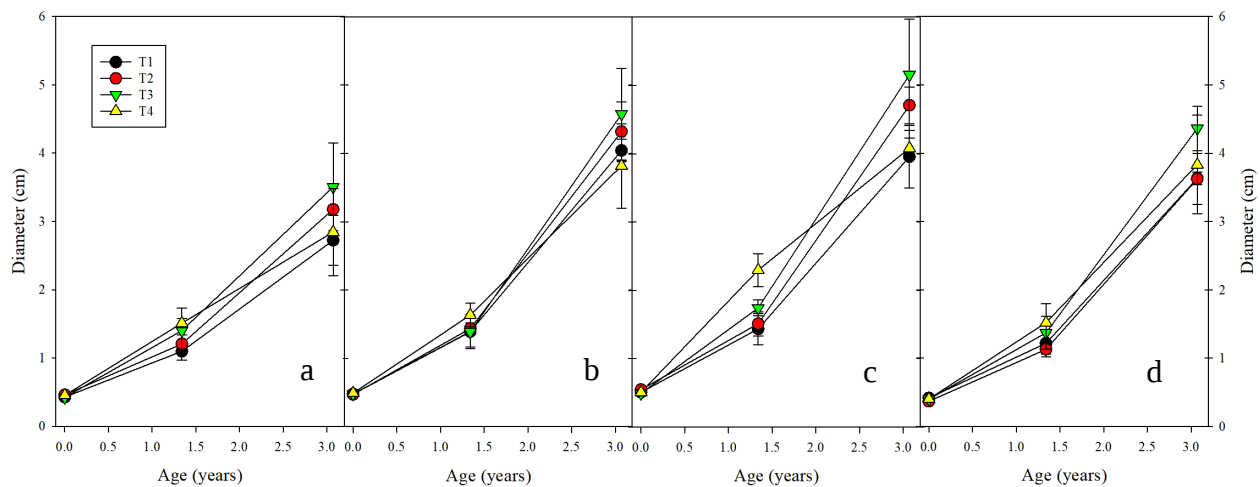


Figure 6. Time series of ground line diameter (cm) for a) *Guazuma crinita*, b) *Eucalyptus x urograndis*, c) *Bixa orellana*, and d) *Psidium guajava* growing under different soil treatments. Error bars represent standard error.

4.4. Stem volume index

The time series of average D^2H for each species and soil treatment are shown in Figure 7. At 3 years after planting, *Eucalyptus x urograndis* showed higher D^2H than *Guazuma crinita* ($P= 0.0141$). Similar to H and D, even though no significant effect was found for soil treatments, a general trend was observed for seedlings growing under T3, having a gain in D^2H of 3.0, 3.1, 4, and 2.3 dm^3 for *Guazuma crinita*, *Eucalyptus x urograndis*, *Bixa orellana* and *Psidium guajava*,

respectively, when compared to seedlings growing in T1 plots (Table 5; Figure 7). That gain corresponds to 142.0, 37.8, 80.0 and 46.9% gain in volume index.

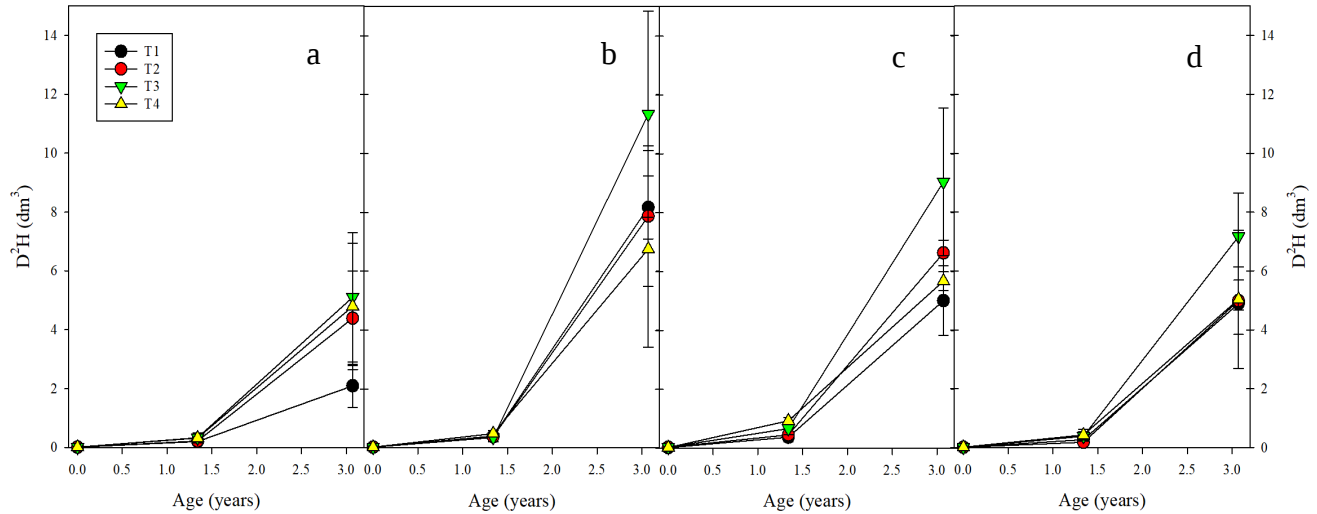


Figure 7. Effect of soil treatments on mean stem volume index (D^2H , dm^3) for a) *Guazuma crinita*, b) *Eucalyptus x urograndis*, c) *Bixa orellana*, and d) *Psidium guajava* over time. Error bars represent standard error.

5. Discussion

The species used in this study showed differences in growth and survival, indicating distinct adaptations to site conditions. Even though no significant effect of soil amendment treatments was detected, a marked trend of positive effect on seedling growth by biochar enriched treatment (T3) was observed. The synergistic effect of T3, which includes biochar mixed with efficient microorganisms, NPK granular, and organic liquid fertilizer, could have a long-term beneficial effect on H, D and D^2H growth. No effect on survival was observed by any soil treatments.

5.1. Seedling survival

Several factors may affect seedling survival during first growing season after planting, including lack of hardening during nursery stage, poor planting, animal damage and extreme weather conditions (frost and/or drought). Also, the differences in survival rates among species may be related to the characteristics of each species and to the environmental conditions of the

site. In our study we observed significant differences across species in seedling survival, where *Psidium guajava* showed higher survival (63.7%) than the other three species (23.3% - 35.6%). This result could be linked to a better resistance to dry periods since *Psidium guajava* is classified as moderately tolerant to drought and water deficit stress (Fischer & Melgarejo, 2021). However, there is little documented information on the best climate and soil conditions for its cultivation (Omayio et al., 2019). *Eucalyptus x urograndis*, showed higher mortality in the first evaluation period (November 2018 - March 2019) when compared to *Psidium guajava* (56.4% and 30.8%, respectively), indicating a lower degree of drought tolerance of the eucalyptus hybrid, a characteristic mentioned by Lopez et al., 2014. Similarly, high mortality was also observed for *Guazuma crinita*, a species that naturally grows in sites with high rainfall (1800 to 2500 mm year⁻¹; ITTO, n.d). The 35.6% survival observed for *Bixa orellana* is much higher than the 1.9% survival reported by Martins et al. (2020), both in areas degraded by mining.

Some species are more resistant to drought while others are not. Coarse-texture soils, as the soil of our study site, are characterized by a reduced soil water availability, having larger potential for increased water stress under low rainfall and high evaporative demand conditions. Papagiannopoulou et al. (2017) pointed out that forest areas seem to be mainly controlled by water availability, either as rainfall and soil moisture, in addition to limiting factors such as temperature, vapor pressure deficit and radiation. Although Papagiannopoulou et al. (2017) mentioned that these factors may be less significant in tropical forests, McDowell et al. (2017), when conducting an extensive review of the factors influencing tree mortality in humid tropical forests, concluded that tree mortality is associated with increased temperature and drought. Therefore, further research along these lines could provide more evidence of the effect of climate changes in tropical ecosystems.

That lack of response in seedling survival to soil treatments agrees with the findings reported by de Farias et al. (2016) and Lefebvre et al. (2019), who evaluated the effects of biochar on growth and survival of different native and non-native tree species planted in degraded areas of the Amazon. According to our study, the survival results lower than 40% of *Eucalyptus x urograndis* with biochar treatments (T2 and T3) could have similarities with the findings of Farias et al. (2016), who found no significant effect on survival under the application of different doses of biochar compared to the non-application during four years of growth.

Our soil amendment treatments indicate no significant effects on the survival values of the four tree species evaluated allowing us to focus efforts on alternatives to improve these results by thinking about limiting factors that are unfavorable to plant survival (i.e., water stress, soil, and climate condition). Further research is required to evaluate other methods or mixtures of these that may favor a higher survival rate of tree species in areas degraded due mining, which may include plant quality, substrates, planting techniques, fertilizers, biochar doses, organic matter addition, and hydro-retainers.

5.2. Seedling growth

At age 3 years, *Eucalyptus x urograndis* seedlings were the biggest (H, D and D²H) of the four species tested in this study. Several *Eucalyptus* species have shown superior growth because its ability to adapt to a broad range of environment conditions (tropical region included) (Kullan et al., 2012). Even though no significant effect was found for soil amendment treatments, a general trend was observed: seedlings growing with enriched biochar addition trend to be bigger. This result agrees with Lefebvre et al. (2019), who reported larger growth of seedlings with biochar plus fertilizers addition. The positive effect of enriched biochar may be related to the synergistic benefits of biochar + efficient microorganisms + organic liquid fertilizer + NPK granular fertilizers

on soils with low fertility and low organic matter content. The effect of biochar applied may be linked to its ability increase nutrient retention and availability due its capacity to prevent nutrient leakage (Glaser et al., 2002 and Laird et al., 2010). In a similar study, Román-Dañobeytia et al. (2020), concluded that species with medium to low wood density can increase growth with biochar additions at planting. Additionally, the efficient microorganisms may not only have benefited the physical, chemical, and biological soil condition but could also influenced plant growth through the ability to produce organic acids and plant hormones (Singh et al.,2011, Biopunto, 2020, and Araujo et al.,2021).

It is important to point out that the soil of the study site, according to soil analysis by CINCIA, has a sandy texture, low organic matter content (ranging from 0.03 to 0.34%), low pH (ranging from 4.78 to 5.86) and presence of Hg. These poor soil conditions, characteristic of soils degraded by mining, could reduce the growth of planted trees (Ferraz, 1993). Low organic matter content may reduce the formation of stable aggregates, porosity, aeration, and water availability for the plant add to that acidic soils would limit the availability of nutrients like phosphorus (Liegel, 2021). Acidic soil also controls the solubility, mobility, and toxicity of heavy metals such as Al, Fe, Mn, Cu, and Zinc (USDA, 2015). Some heavy metals function as micronutrients (e.g., Cu, Fe, Mn, Mo, and Zn) important for plant physiological function (Goyal et al., 2020). However, there are heavy metals that are not vital for plant growth such as Pb, As, Cd, Hg (Asati et al., 2016 and Goyal et al., 2020). High levels of these heavy metals as is the case of Hg, widely reported in the Peruvian Amazon due mining activities, could have produced a toxicity effect on planted plants. These effects may include damage to cellular structures, reduction of photosynthetic activities and mineral nutrition of the plant affecting its growth and development (Asati et al., 2016 and Goyal et al., 2020). Román-Dañobeytia et al. (2015) reports that accumulation of Hg can be found in

various types of tissues of tree species (root, leaves, and stems) after being planted in abandoned gold mines.

At the study site, in addition to the above mentioned, frequent rainfall and lack of vegetative cover could have led to water erosion, increasing surface runoff, and leaching of soil nutrients. Physical, biological, and chemical indicators are sensitive to soil management, so organic and inorganic amendments could change these soil indicators (USDA, 2015). In addition, to the synergistic effect of biochar (T3) enrichment in favoring the growth and development of studied species other alternatives could be included as soil amendments. For example, studies by Martínez-Fernández et al. (2014) show that in soils contaminated with heavy metals, the addition of organic matter and microorganisms, in addition to increasing soil fertility and plant biomass, could reduce the concentration of trace elements in plant species. Hence, the inclusion of different types of soil amendments and/or the combination of soil amendments to improve soil productivity could also be considered as remediation/restoration strategies in further research.

These results could be extended for the evaluation of biomass production capacity or productivity of these species on similar sites. Although *Eucalyptus x urograndis* showed larger D^2H than the other species, it is interesting to highlight that all species used in this study could attain higher growth with the addition of enriched biochar at planting. Due to its ability to immobilize heavy metals and possible promotion of the availability of other nutrients (Shue et al., 2016 and Yang et al., 2021), biochar in post-mining sites could work for soil amending in remediation projects. Although alternatives are being explored to increase seedling productivity, growth rates are still marginal in areas degraded by mining (Roman-Dañobeytia et al., 2015). So, remediation and restoration projects in the Peruvian Amazon are still highly challenging.

6. Conclusion

This study shows how species selection and the use of soil amendments used in planting practices can have a strong influence on the reforestation of areas degraded by mining in the Peruvian Amazon. The survival analysis showed strong differences across the four species tested (*Guazuma crinita*, *Eucalyptus grandis x urophylla*, *Bixa orellana*, and *Psidium guajava*), but no effect of the soil amendment treatments applied. Our results agree with the findings of multiple authors. Regarding the analysis of the effects of soil amendment treatments on seedling growth, even though the improvement of soil conditions based on increasing nutrients and water availability could generate positive effects, no significant response was observed, but only a trend of increased growth by the application of enriched biochar aroused in age three years measurement.

Our results improve our understanding of the survival and growth responses of *Guazuma crinita*, *Eucalyptus grandis x urophylla*, *Bixa orellana*, and *Psidium guajava* planted in areas degraded by mining under different planting treatments. This could be considered as part of a strategic guideline to implement remediation activities using local resources. Dumroese et al. (2016) recommend that, in order to meet challenges of restoration, the target plant concept must be understood. Priority should be given to species selection, source-identified seeds, appropriate container type and size, and nursery cultural practices to obtain seedlings with specific characteristics (height and stem diameter) to different field conditions. Recognizing appropriate seedling target characteristics may depend on site conditions that drive the levels of stresses that the seedlings could experience.

Further research extending the observation period is required before any final recommendation about the treatments evaluated in the study. The continuous search for improving reforestation strategies is necessary when recovering forest areas, for increasing monetary value (timber and

non-timber products) and the sustainability of the environment and livelihoods of local communities.

7. Recommendations

- Continue investigating alternatives to increase survival for reforestation of degraded areas in the Amazon, focusing mainly in soil moisture retention and soil & plant water relations.
- Additional studies are required to generate more evidence on the effect of enriched biochar on plant growth when installed in areas degraded by mining. Identifying the differentiated effect of each of its components could help direct efforts to optimize resources and results.
- Assessing the content of heavy metals in plant tissue could be useful in analyzing the remediation effect of biochar.
- Consider the target plant concept to define the main characteristics of seedlings according to the planting site.
- Increase capacity building of local community members in order to improve the success of any future reforestation project.

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